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117- POST-HARVEST APPLICATION OF BACTERIOPHAGES ON BEEF AS A NATURAL INTERVENTION AGAINST E. COLI O157

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Objectives: Despite the use of chemical interventions in a multi-hurdle approach against *Escherichia coli* O157, this pathogen remains a great concern for the beef industry. Bacteriophages are the natural enemy of bacteria, and able to specifically kill *E. coli* O157, for example. Together with the fact that phages are harmless to human, phages have the potential to form an additional safe and effective intervention against *E. coli* O157.
The objective of this study was to determine the efficacy of bacteriophages as natural intervention against *Escherichia coli* O157 on refrigerated beef.
Materials and Methods: Bacteriophages were isolated from sewage water, after which lysis activity of the isolated phages was assessed by spotting serial dilutions on 88 *E. coli* O157 strains. For efficacy assays on beef, two cold (4 °C/39.2 °F) beef cuts of 9 cm^2 were contaminated per treatment with *E. coli* O157 at a rate of 1 x 10^5 cfu/cm^2. Subsequently, samples were treated with 3 x 10^7 or 3 x 10^8 Plaque Forming Units (PFU)/cm^2 by applying 5 µL/cm^2 of a cocktail containing two selected phages. Controls were treated with similar volumes of tap water. Beef samples were then incubated for 24 hours at 4 °C, after which bacteria were retrieved for enumeration. Bacterial reductions on phage treated samples were calculated relative to tap water treated controls. Reductions of four different *E. coli* O157 strains obtained in three independent experiments were used for statistical analysis (Unpaired t-test).
Two separate time trials were done in which beef samples were contaminated and treated as described above. In one time trial, beef samples were stored at 4 °C during the complete experiment. In the second time trial, all samples were initially stored at 4 °C for 24 hours, after which all remaining samples were transferred to an abusive temperature of 20 °C (68.0 °F) for the remainder of the experiment. For both time trials, two beef samples per treatment were retrieved for bacterial enumeration at 2, 6, 24, 30, 48, and 54 hours post phage treatment. Results were confirmed in three independent experiments on which statistical analysis per time point was done (Unpaired t-test).
Results: After assessing the lysis activity of the isolated phages on 88 *E. coli* O157 strains, two phages were selected that showed a complementing hostrange activity, lysing 90 % of all *E. coli* O157 strains tested. A cocktail of the two selected phages showed bacterial reductions between 1.5 -1.9 log_{10} ($P<0.05$) when cold beef was treated with 3 x 10^8 PFU/cm^2, while 0.8 – 1.5 log_{10} ($P<0.05$) reductions were observed with 3 x 10^7 PFU/cm^2. In both time trials, we observed that the maximum reduction was already achieved 6 hours post phage application. Furthermore, in the first time trial at 4 °C, we observed no further reduction in bacterial load after the 6 hours time point (1.25 – 2.25 Log_{10} reduction at all time points, $P<0.05$). Transferring the treated samples to an abusive temperature of 20 °C, resulted in outgrowth of the remaining *E. coli* O157 at a similar rate as the non-treated controls.
Conclusion: The two phage cocktail described above significantly reduces *E. coli* O157 on refrigerated beef. Furthermore, the time trials show that phages work fast on cold beef, and that they can be regarded as processing aid. All in all, we show that bacteriophages provide a natural, safe, and effective intervention for the beef industry to fight *E. coli* O157.
Keywords: Bacteriophage, Beef, E. coli O157, Intervention, Phage

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118-SYNERGISTIC EFFECT OF PHAGES AND ORGANIC ACID SALTS ON LISTERIA CONTAMINATED READY TO EAT TURKEY HAM

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Objectives: Ready To Eat (RTE) are foods that have already been cooked and needs no further heating, making it easy and fast for consumers to prepare meals. For this reason that most RTE do not undergo any lethality steps at consumer level before consumption, companies must care to deliver "*Listeria*-free" foods.
In this study ability of PhageGuard Listex (a natural bacteriophage solution against *Listeria*) in combination with buffered vinegar or a commercially available solution comprising potassium lactate (72.8%) and sodium diacetate (5.2%), to control *Listeria* during the shelf-life (120 days) of sliced RTE turkey meat was assessed.
Materials and Methods: Cooked turkey slices were inoculated with a cocktail of four *Listeria monocytogenes* strains at 1 Log cfu/cm^2 (duplicate sample per treatment). Contaminated samples were treated by spraying either only PhageGuard Listex (2x10^7 PFU (Plaque Forming Units)/cm^2), PhageGuard Listex plus buffered vinegar and PhageGuard Listex plus potassium lactate/sodium diacetate on the surface of the turkey slices. Other samples were sequentially treated with just either of the organic acids and tap water (untreated) and were used as negative controls. Subsequently, all samples were vacuum packed and stored at 40°F/4°C. During the shelf-life of 120 days, samples were retrieved in peptone buffered water to detect and enumerate *Listeria*.
Results: When applied on artificially contaminated turkey ham, the phage solution or buffered vinegar alone kept *Listeria* concentration below detection limit for 20 and 30 days respectively (mean value of two individual experiments). However, when a combination of phages with buffered vinegar was applied, *Listeria* was kept below detection limit for 120 days (mean value of two individual experiments). A similar though less strong, effect was observed when turkey slices were sequentially treated with a combination of phages and potassium lactate/sodium diacetate. In this case, *Listeria* was not detected for 30 days (mean value of two individual experiments) and *Listeria* was kept below 2 logs outgrowth (compared to the initial contamination) for 70 days (mean value of two individual experiments). Application of just potassium lactate/sodium diacetate alone resulted in more than 2 logs outgrowth after 40 days.
Conclusion: A clear synergistic effect was observed when combining a bacteriophage product against *Listeria* with two different organic acid solutions on *Listeria* contaminated cooked turkey slices. Results showed combining phages with buffered vinegar, *Listeria* levels were kept below detection limits for an additional 90 days when compared to buffered vinegar alone. Combining such *Listeria*-specific phage product with these organic acids has the added beneficial effect of inhibiting the outgrowth of spoilage bacteria.
All in all, these results establish the combined effect of PhageGuard Listex and organic acids as an effective anti-listeria hurdle during processing of RTE meals, leading to an increase in consumer safety.
Keywords: Listeria, organic acids, phage, RTE, turkey ham

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139- EFFICACY OF A COMMERCIAL PHAGE COCKTAIL IN REDUCING SALMONELLA CONTAMINATION ON POULTRY PRODUCTS - LABORATORY DATA AND INDUSTRIAL TRIAL DATA

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Objectives: We endeavored to test the efficacy of two phages that show an extremely broad host range in *in vitro* studies on poultry products using a) artificial contamination studies and b) by assessing their usefulness in factory settings with naturally contaminated products.
Materials and Methods: The commercial *Salmonella* phage product PhageGuard S was used in these experiments. For the artificial contamination, poultry products (skin-on and skinless) were purchased from local stores. A streptomycin resistant *Salmonella*. Enteritidis strain was used to contaminate both products with ~10^4 CFU/cm^2. Phage was applied at 1 and 2 x10^7 pfu/cm^2 after 30 min. Bacteria were retrieved from treated and control samples after 24, 48 and 144 hours. Duplicate samples were used and experiments were repeated twice. After stomaching dilutions were spread on *Salmonella* selective agar plates supplemented with streptomycin to further reduce background flora. In the tests in industrial trials phages were applied by dipping the products in phage solutions containing 1-4% of the phage product resulting in similar numbers of phages as used in the artificial contamination studies. All food products were tested twice. Experiments were performed off-line allowing comparison with regular non-treated product. In these trials absence/presence in a given sample was recorded using the detection methods preferred by the respective establishment. Different poultry producers were involved in the trials which were performed on-site. In short chicken livers, necks and breasts as well as turkey backs were treated and absence/presence of *Salmonella* was established according to the establishments (USDA-approved) testing method and compared to an equal number of untreated control samples (180, 120, 450 and 74 tested samples in total respectively).
Results: Both on skinless and skin-on poultry products phage application resulted in >1 log reductions for both phage concentrations used. The reduction in cells did not increase after 24 hours indicating that phage are active for a short period of time. In the industrial trials 19/90 untreated chicken liver samples were positive (21%) whereas in the treated group 1/90 samples was (~1%). 50/60 untreated neck samples were found to be positive (83%) whereas in the treated samples 21 were positive. In the chicken breast trial 49% of the control samples (94/190) were positive and 13% of treated samples were (33/260). For the turkey backs all 37 control samples were positive whereas only 8 on the treated samples were. This translates in reduction of positive samples by 94%, 58%, 80% and 88% respectively. Analysis using Fischers' exact test shows that the differences are statistically significant.
Conclusion: The results from the artificial contamination experiments show that phage can significantly reduce Salmonella on poultry products. The results from the industry trials show that this translates into meaningful reductions in real life and that phages offer a valuable new tool to enhance food safety.
Keywords: Bio-control, Field trials, Phage, Salmonella

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140- EFFICIENCY OF PHAGE INTERVENTION ON SALMONELLA KILL ON LEAN PORK, PORK TRIM AND BACON

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Objectives: Raw Pork Products Exploratory Sampling Program (2015) by FSIS, USDA aims to estimate the prevalence and levels of *Salmonella* in various fresh pork cuts and products. The results of this ongoing baseline sampling program will provide direction to FSIS and the Agency to develop a better risk profile and refine the present food safety guidelines for pork products. New impending regulatory standards will likely prompt establishments to seek interventions that can help in reducing the prevalence and most probable number of *Salmonella* in their products.
Bacteriophages are ubiquitous antibacterial agents that can invade and kill specific target pathogenic bacteria in foods. PhageGuard S, is a commercial salmonella based phage cocktail consisting of 2 phages, FO1a and S16. Having been found in nature, these phages are a natural and organic way to kill salmonella and reduce risk in various types of food products. Phage have a number of other added benefits over traditional chemical processing aids as well. There is no impact to the sensorial properties of foods and no impact on worker safety, wastewater or processing equipment. As such, the use of *Salmonella*-phages as a biocontrol agent for *Salmonella* can be an interesting avenue to explore for the meat industry.
This study determines the efficacy of a commercially available phage product, PhageGuard S, for *Salmonella* kill on several types of pork including – lean meat, bacon and pork trim.
Materials and Methods: Overnight culture of *Salmonella* Se13 streptomycin resistant strain was diluted and inoculated at a concentration of $2x10^4$ CFU/cm^2 or CFU/g on lean pork, bacon or pork trims (duplicate samples per treatment). Subsequently, contaminated samples were treated with either of the following phage concentrations ($5x10^6$,$1x10^7$,$2x10^7$ or $5x10^7$ PFU/cm^2 or PFU/g) or water (control). After treatment, samples were stored at 40 °F (4 °C) for 18 hours before retrieval and enumeration of bacteria on selective agar plates.
Results: The application of phages 10^7 and $5x10^7$ PFU (Plaque Forming Unit)/cm^2 on lean pork resulted in 1.1 $\log_{10}$ CFU/cm^2 and 1.6 $\log_{10}$ CFU/cm^2 reduction of *Salmonella* respectively (mean value of two individual experiments). On Bacon, the highest phage concentration showed *Salmonella* reduction of 1.3 $\log_{10}$ CFU/cm^2 and the lowest phage concentration resulted in 0.8 $\log_{10}$ CFU/cm^2 reduction (mean value of two individual experiments). When applied on pork trim, phage concentration $2x10^7$ and $5x10^7$ PFU/g showed *Salmonella* kill of 1.3 $\log_{10}$ CFU/g and 1.7 $\log_{10}$ CFU/g respectively. Overall, a dose response was observed where increasing phage concentration resulted in an increasing *Salmonella* kill on different pork meat.
Conclusion: Phage technology is an easy, safe intervention and an alternative to chemicals and antibiotics in controlling of *Salmonella* in slaughter and processing environments. The above results indicate that the commercially available phage solution, PhageGuard S, can reduce *Salmonella* contamination on pork by 1.3 to 1.7 $\log_{10}$. Therefore, making it an effective *Salmonella* intervention for processors to reduce risks and allow for increase in consumer safety.
Keywords: bacon, Bacteriophage, pork, Salmonella, trim